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The effects of short term, long term and reapplication of biochar on soil bacteria

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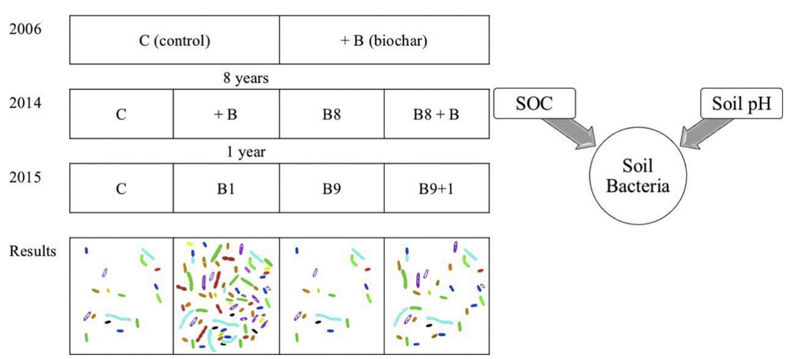
HIGHLIGHTS

- Soil bacterial diversity significantly increased with 1-year biochar amendment.
- Nitrifiers and bacteria decomposing pyrogenic C were enriched in 1-year amendment.
- Bacterial structure under 9-year and repeated amendments did not differ from control.
- SOC and pH affected bacterial community in recently applied biochar amendment.

GRAPHICAL ABSTRACT



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ABSTRACT

Biochar has been shown to affect soil microbial diversity and abundance. Soil microbes play a key role in soil nutrient cycling, but there is still a dearth of knowledge on the responses of soil microbes to biochar amendments, particularly for longer-term or repeated applications. We sampled soil from a field trial to determine the individual and combined effects of newly applied (1 year ago), re-applied (1 year ago into aged biochar) and aged (9 years ago) biochar amendments on soil bacterial communities, with the aim of identifying the potential underlying mechanisms or consequences of these effects. Soil bacterial diversity and community composition were analysed by sequencing of 16S rRNA using a Miseq platform. This investigation showed that biochar in soil after 1 year significantly increased bacterial diversity and the relative abundance of nitrifiers and bacteria consuming pyrogenic carbon (C). We also found that the reapplication of biochar had no significant effects on soil bacterial communities. Mantel correlation between bacterial diversity and soil chemical properties for four treatments showed that the changes in soil microbial community composition were well explained by soil pH, electrical conductivity (EC), extractable organic C and total extractable nitrogen (N). These results suggested that the effects of biochar amendment on soil bacterial communities were highly time-dependent. Our study highlighted the acclimation of soil bacteria on

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receiving repeated biochar amendment, leading to similar bacterial diversity and community structure among 9-years old applied biochar, repeated biochar treatments and control.

1. Introduction

Biochar is a carbon (C) rich by-product of biomass pyrolysis and is produced at relatively low temperatures (<700 °C) without oxygen (O_2) (Lehmann and Joseph, 2009). Determining the biochar-induced changes in soil microbial communities can provide insights into how biochar affects soil fertility since many processes of nutrient cycling are mediated by microbes (Anderson et al., 2011; Kolton et al., 2011; Joseph et al., 2016). Biochar application may affect C and N cycling in the soil-plant-microbe system (Nguyen et al., 2017a, 2017b; Zhang et al., 2017a, 2017b; Zhang et al., 2018). The porous nature of biochar provides niches for colonisation of soil microorganisms (e.g., bacteria, fungi, and protozoa) and protects them from predators (Zackrisson et al., 1996; Warnock et al., 2007; Quilliam et al., 2013). Recent meta-analyses have shown that pH of biochar and the properties of labile organic compounds in biochar are crucial factors affecting microbial growth on biochar surfaces (Gul et al., 2015; Nguyen et al., 2017a). Changes in soil pH and organic compounds can affect microbial community composition, for example, Gram-positive bacteria prefer higher pH and biochar-derived C (Santos et al., 2012; Farrell et al., 2013) whereas Gram-negative bacteria prefer lower pH and easily degradable organic compounds (Gul et al., 2015).

In the short term, biochar stimulates growth of microorganisms involved in C and nitrogen (N) cycling (Anderson et al., 2011; Harter et al., 2014; Xu et al., 2014; Chen et al., 2015) and affects C and N dynamics in the soil (Darby et al., 2016; He et al., 2016; Bai et al., 2015; Nguyen et al., 2017a). For example, biochar from *Pinus radiate* increased the abundance of bacterial groups involved in C and N cycling by 11% compared with the control after 12 weeks of a pot trial (Anderson et al., 2011). Also, the abundances of photo/chemotroph microbes using both labile and aromatic C were increased in biochar-amended soil 12 weeks after application (Im et al., 2006; Anderson et al., 2011). Increased microbial abundance of N_2-fixing and denitrifying *Bradyrhizobium* bacteria has been attributed to increased pH of acid soil, aeration, and improved water holding capacity after biochar application in a pot experiment (Anderson et al., 2011).

There have been some long-term studies of effects of biochar on soil microbes (Kuzyakov et al., 2009; Khodadad et al., 2011; Anderson et al., 2014). For example, effects of biochar on soil microbes have been evaluated after 2 years (Solaiman et al., 2010; Anderson et al., 2014), 2.5 years (Khodadad et al., 2011), 3 years (Quilliam et al., 2012; Rousk et al., 2013; Spokas, 2013), and 3.2 years (Kuzyakov et al., 2009). Most of those studies reported effects of biochar on microbial biomass C and N, and bacterial and fungal growth rates but not microbial diversity and community structure. Alternatively, some studies assessing shifts in soil microbial structure have observed no significant influence of aged biochar on soil microbial community structure when biochar remained in soil for 2 (Anderson et al., 2014) or 3 years (Quilliam et al., 2012). Mechanisms of those effects also remain unclear.

The effects of multiple biochar applications have been rarely investigated because the short-term effects of biochar on soil microorganisms involved in nutrient cycling have usually been assessed with single applications (Quilliam et al., 2012). Up to date, only one study has published investigating the effects of repeated biochar application on soil microbes when biochar re-application occurs after 3 years of the first application at low and high rate ($25 + 25$ t ha^{-1} and $50 + 50$ t ha^{-1}) (Quilliam et al., 2012). However, they did not find any significant change of soil microbial diversity in 60 days following biochar re-application (Quilliam et al., 2012). Besides, most existing studies so far on the impact of biochar on soil microbial communities are laboratory incubation or glasshouse experiments, whilst the processes in the field may differ due to the effects of location, climate, animals, and humans (Anderson et al., 2011; Kolton et al., 2011; Nielsen et al., 2014).

In this study, we investigated the effects of field-aged biochar and repeated biochar application on soil microbial community diversity and community structure in a field trial. We also assessed soil chemical properties to explore possible mechanisms driving the shifts of these microbial communities. Better understanding the effect of long-term and reapplied biochar application on soil microbes can help interpreting biochar effects on C and N processes which are mainly mediated by soil microbes. Our study aimed to examine whether the effects of biochar on soil bacteria including diversity and particularly groups involved in C and N are transient. We hypothesised that (1) short-term biochar application would have greater effect on soil microbial community structure compared with aged and repeated biochar treatments due to rapid changes in soil pH, C and N availability, and (2) short-term biochar application would promote bacteria involved in N cycling through improved soil organic C and pH.

2. Materials and methods

2.1. Site description

The experimental site is located on a highly permeable red Ferralsol derived from Tertiary basalt (Nicholls et al., 1953) at the Wollongbar Primary Industries Institute (28°49′S, 153°23′E; elevation 140 m), Wollongbar, New South Wales, Australia. The experimental site is located in a subtropical zone with most precipitation in late spring to summer (November–February). The annual rainfall was ranged from 46 to 398 mm, and the annual temperature was ranged from 6.5 to 20.6 °C. The site was converted from subtropical forest to dairy pasture and managed as an intensive diary pasture (Weng et al., 2015). The details of soil properties are provided in Table 1.

2.2. Treatments and experimental design

2.2.1. Biochar

The feedstock of biochar was from a single-source, homogeneous whole-tree residue (Eucalyptus saligna). The hardwood residue was

Table 1
Physiochemical properties of soil and greenwaste biochar (van Zwieten et al., 2010; Slavich et al., 2013).

Analysis	Units	Soil	Fresh GW biochar	9-Year biochar
Total C	%	4.5	76	54
Total N	%	0.28	0.22	0.4
C:N			345	137
EC	dS m^{-1}	–	0.14	–
pH($CaCl_2$)		4.5	7.8	–
Acid neutralising capacity	% $CaCO_3$	–	5.6	–
Total P	mg kg^{-1}	1020	190	–
P-Bray1	mg kg^{-1}	510	6	–
NH_4^+-N	mg kg^{-1}	3.7	<0.3	–
NO_3^--N	mg kg^{-1}	180	<0.2	–
CEC	cmol (+) kg^{-1}	6.2	1.2	–
Total S	%	0.0235	0.0078	–
Total Fe	%	8.4	–	–
Total Al	%	6.7	–	–